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0300

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(MBHB00-884-H; 500/001)

In re the Application of:)
)
Breaker, et al.) **Group Art Unit: 1645**
)
) **Examiner: Unassigned**
Serial No.: 09/780,929)
)
Filed: February 8, 2001)
)
For: Nucleic Acid Catalysts with Endonuclease)
Activity)

AMENDMENT AND RESPONSE TO THE NOTICE TO COMPLY
MAILED September 7, 2001

Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant respectfully request that the following amendments to the claims, specification, and figures be entered in response to the Notice to Comply mailed on September 7, 2001.

AMENDMENT

IN THE CLAIMS:

Please amend the claims as follow:

2. (Amended) A nucleic acid molecule with endonuclease activity having the formula II:



CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington D.C. 20231, on November 7, 2001

Date: November 7, 2001


Linda J. Atkins Duffy

wherein, N represents a nucleotide or a non-nucleotide linker; X and Y are independently oligonucleotides of length sufficient to stably interact with a target nucleic acid molecule; Z is an oligonucleotide having a nucleotide sequence selected from the group consisting of 5'-AGAUAACGUGAAGAU-3' (SEQ ID NO 97) and 5'-AAUGGCCUAUCGGUGCGA-3' (SEQ ID NO 98); _____ represents a chemical linkage; and C, G, A, and U represent cytidine, guanosine, adenosine and uridine nucleotides, respectively.

A marked up copy of the original paragraph showing the changes made by this amendment is attached as **APPENDIX A**.

IN THE SPECIFICATION:

Please replace the paragraph found on page 4, line 7 – page 5, line 10 with the following:

“ In the above formulae, each N represents independently a nucleotide or a non-nucleotide linker, which may be same or different; X and Y are independently oligonucleotides of length sufficient to stably interact (e.g., by forming hydrogen bonds with complementary nucleotides in the target) with a target nucleic acid molecule (the target can be an RNA, DNA or RNA/DNA mixed polymers, including polymers that may include base, sugar, and/or phosphate nucleotide modifications; such modifications are preferably naturally occurring modifications), preferably, the length of X and Y are independently between 3-20 nucleotides long, e.g., specifically, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, and 20); X and Y may have the same lengths or may have different lengths; m, n, o, and p are integers independently greater than or equal to 1 and preferably less than about 100, specifically 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 50; wherein if (N)_m and (N)_n and/or (N)_o and (N)_p are nucleotides, (N)_m and (N)_n and/or (N)_o and (N)_p are optionally able to interact by hydrogen bond interaction; preferably, (N)_m and (N)_n and/or (N)_o and (N)_p independently form 1, 2, 3, 4, 5, 6, 7, 8, 9 base-paired stem structures; D is U, G or A; L₁ and L₂ are independently linkers, which may be the

same or different and which may be present or absent (*i.e.*, the molecule is assembled from two separate molecules), but when present, are nucleotide and/or non-nucleotide linkers, which may comprise a single-stranded and/or double-stranded region; _____ represents a chemical linkage (*e.g.* a phosphate ester linkage, amide linkage or others known in the art); • represents a base-pair interaction; Z is independently a nucleotide sequence selected from the group comprising 5'-AGAUAACGUGAAGAU-3' (SEQ ID NO 97) and 5'-AAUGGCCUAUCGGUGCGA-3' (SEQ ID NO 98), additions, deletions, and substitutions to these sequences may be made without significantly altering the activity of the molecules and are hence within the scope of the invention; and C, G, A, and U represent cytidine, guanosine, adenosine and uridine nucleotides, respectively. The nucleotides in each of the formulae I and II are unmodified or modified at the sugar, base, and/or phosphate as known in the art. ”

Please replace the paragraph on page 11, lines 26-28 with the following:

“ **Figure 7** is a representative diagram of structural similarities between Class I (SEQ ID NO 117), (substrate SEQ ID NO 103) and Class VIII (SEQ ID NO 121), (substrate SEQ ID NO 103) enzymatic nucleic acid molecules of the invention. ”

Please replace the paragraph on page 39, lines 2-15 with the following:

“ The initial population of RNA for *in vitro* selection was created by first generating a double-stranded DNA template for *in vitro* transcription. SuperScript® II reverse transcriptase (RT, GibcoBRL) was used to extend 280 pmoles of the DNA oligonucleotide “primer 1” (5'-GAAATAAACTCGCTTGGAGTAACCATCAGG-ACAGCGACCGTA-3') (SEQ ID NO 99); region representing 16 possible nearest neighbor combinations is underlined) using 270 pmoles of the template DNA (5'-TCTAATACGACTCACTATAGGAAGACGTAGCCAAN₄₀TACGGTCGCTGTCC TG-3') (SEQ ID NO 100); T7 promoter is underlined and N represents an equal

mixture of the four standard nucleotides). The extension reaction was conducted in a total of 50 μ l containing 50 mM Tris-HCl (pH 8.3 at 23°C), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol (DTT), 0.2 mM each of the four deoxyribonucleoside-5' triphosphates (dNTPs), and 10 U μ l⁻¹ RT by incubation at 37°C for 1 hr. The resulting double-stranded DNA was precipitated by the addition of 5 μ l 3 M sodium acetate (pH 5.5) and 140 μ l 100% ethanol and pelleted by centrifugation. This extension reaction provides $\sim 10^{14}$ different template sequences."

Please replace the paragraph on page 39, line 27 – page 40, line 10 with the following:

"The initial selection reaction (G0) contained 2000 pmoles of RNA in a total of 400 μ l reaction buffer (50 mM HEPES [pH 7.5 at 23°C], 250 mM KCl and 20 mM MgCl₂) and was incubated at 23°C for 4 hr. The reaction was terminated by the addition of EDTA and the RNA was recovered by precipitation with ethanol. RNA cleavage products were separated by denaturing 10% PAGE, visualized and quantified using a Molecular Dynamics PhosphorImager®, and the gel region corresponding to the location of the desired RNA cleavage products was excised. The RNA was recovered from the excised gel by crush-soak elution followed by precipitation with ethanol. The selected RNAs were amplified by RT-PCR as described previously (10) using primers 1 and 2 (5'-GAATTCTAATACGACTCACTATAGGAA-GACGTAGCCAA-3') (SEQ ID NO 101); T7 promoter is underlined). The resulting double-stranded DNA from each round of *in vitro* selection was used to transcribe the RNA population for the subsequent round, in which all steps were conducted at $\sim 1/10^{\text{th}}$ the scale of G0. All other parameters of the selection process were maintained as in G0. Representative ribozymes from the populations derived from 6, 9, 12 and 15 rounds of selection were examined by cloning (TOPO®-TA cloning kit; Invitrogen) and sequencing (ThermoSequenase® kit; Amersham Pharmacia)."

A marked up copy of the original paragraph showing the changes made by this amendment is attached as **APPENDIX B**.

IN THE TABLES:

Please replace the originally filed Tables VII and VIII (pages 53-55, as filed), with substitute Tables VII-VIII (pages 53-55), submitted herewith.

THE SEQUENCE LISTING:

Please incorporate the sequence listing submitted herewith on paper into the application after the Abstract (page 62), in compliance with CFR § 1.77(b). The sequence listing is submitted herewith in CRF format (3.5" disk) and on paper, in compliance with 37 CFR §§ 1.821-1.825.

The sequences in the sequence listing are consistent with those found in the amended specification, Tables, and Figures, submitted herewith, and do not constitute new matter.

IN THE FIGURES:

Please replace Figures 1-7, with substitute Figures 1-7, submitted herewith.

A marked up copy of the original Figures 1-7 showing changes made by this amendment is attached as **APPENDIX C**.

REMARKS

Claims

Claim 2 has been amended only by changing the SEQ ID NO used to identify the sequences contained in the claim so that they conform with the amendments to the Tables, specification, Figures, and the sequence listing submitted herewith. This amendment has not been made for any reason related to patentability, and does not constitute new matter. A copy of the marked up original claim 2 showing changes made by this amendment is attached as **Appendix A**.

Specification

A. *Specification paragraph:*

The paragraphs on pages 4, 11, 39, and 40 of the specification were amended to provide clarification and to include the appropriate SEQ ID NO, in compliance with 37 C.F.R. § 1.821-1.825. These amendments have merely changed the SEQ ID NOs used to identify the disclosed sequences in order to remove redundant SEQ ID NOs and to conform with the amended Tables and Figures. These amendments do not substantively alter the disclosed subject matter and do not constitute new matter. A copy of the marked up original paragraphs are attached as **Appendix B.**

B. *The Amended Tables:*

The Tables VII-VIII (pages 53-55) of the specification were amended to re-number the SEQ ID NOs for the sequences included therein, and to remove two redundant sequences in Table VII, so that the numbers conform with numbering found in the sequence listing, submitted herewith. Additionally, each sequence disclosed in Table VIII is now identified by a unique SEQ ID NO, in compliance with CFR §§ 1.821-1.825. These amendments do not constitute new matter, and only serve to clarify the invention and bring the application into compliance with the sequence listing rules. New tables VII-VIII are found on substitute sheets pages 53-55, submitted herewith.

No new matter has been added by way of any of these amendments to the specification.

Figures:

Revised Figures 1-7 were amended to include the appropriate SEQ ID NOs in order to identify each disclosed sequence in compliance with 37 C.F.R. § 1.821-1.825. These amendments have been made to clarify further the subject matter of the Figures. No new matter has been added by way of these amendments. A marked-up version of the originally filed Figures 1-7 showing changes made by these amendments are attached as **Appendix C.**

Sequence Listing:

In compliance with 37 C.F.R. §§ 1.821-1.825, the applicant herewith submits the Sequence Listing on paper under 37 C.F.R. § 1.821(c), and computer readable form copy (CRF) on 3.5" disk under 37 C.F.R. § 1.821(e). The content of the paper copy and the computer readable form of the Sequence Listing are the same.

The Sequence Listing has been generated from the specification and does not constitute new subject matter.

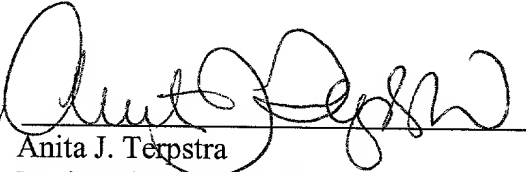
Conclusion

The Commissioner is hereby authorized to charge payment of any fees required in connection with the papers transmitted herewith, or to credit any overpayment of same, to Deposit Account No. 13-2490.

If the Examiner has any questions regarding this Response and Amendment, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,
**McDONNELL BOEHNEN HULBERT
& BERGHOFF**

Date: November 7, 2001

By 
Anita J. Terpstra
Registration No. 47,132

APPENDIX A
Version With Markings to Show Changes Made to Claims

Bold, underline text, **for example**, indicates inserted text
Bracketed, strikethrough text, [~~for example~~], indicates deleted text

IN THE CLAIMS:

2. (Amended) A nucleic acid molecule with endonuclease activity having the formula II:



wherein, N represents a nucleotide or a non-nucleotide linker; X and Y are independently oligonucleotides of length sufficient to stably interact with a target nucleic acid molecule; Z is an oligonucleotide having a nucleotide sequence selected from the group consisting of 5'-AGAUAACGUGAAGAU-3' (SEQ ID NO [53]**97**) and 5'-AAUGGCCUAUCGGUGCGA-3' (SEQ ID NO [54]**98**); _____ represents a chemical linkage; and C, G, A, and U represent cytidine, guanosine, adenosine and uridine nucleotides, respectively.

APPENDIX B
Version With Markings to Show Changes Made to Specification

Bold, underline text, **for example**, indicates inserted text
Bracketed, strikethrough text, [~~for example~~], indicates deleted text

IN THE SPECIFICATION:

The paragraph on page 4, line 7 – page 5, line 10:

In the above formulae, each N represents independently a nucleotide or a non-nucleotide linker, which may be same or different; X and Y are independently oligonucleotides of length sufficient to stably interact (e.g., by forming hydrogen bonds with complementary nucleotides in the target) with a target nucleic acid molecule (the target can be an RNA, DNA or RNA/DNA mixed polymers, including polymers that may include base, sugar, and/or phosphate nucleotide modifications; such modifications are preferably naturally occurring modifications), preferably, the length of X and Y are independently between 3-20 nucleotides long, e.g., specifically, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, and 20); X and Y may have the same lengths or may have different lengths; m, n, o, and p are integers independently greater than or equal to 1 and preferably less than about 100, specifically 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 50; wherein if (N)_m and (N)_n and/or (N)_o and (N)_p are nucleotides, (N)_m and (N)_n and/or (N)_o and (N)_p are optionally able to interact by hydrogen bond interaction; preferably, (N)_m and (N)_n and/or (N)_o and (N)_p independently form 1, 2, 3, 4, 5, 6, 7, 8, 9 base-paired stem structures; D is U, G or A; L₁ and L₂ are independently linkers, which may be the same or different and which may be present or absent (i.e., the molecule is assembled from two separate molecules), but when present, are nucleotide and/or non-nucleotide linkers, which may comprise a single-stranded and/or double-stranded region; _____ represents a chemical linkage (e.g. a phosphate ester linkage, amide linkage or others known in the art); • represents a base-pair interaction; Z is independently a nucleotide sequence selected from the group comprising 5'-AGAUAACGUGAAGAU-3' (SEQ ID NO [53]**97**) and 5'-AAUGGCCUAUCGGUGCGA-3' (SEQ ID NO [54]**98**), additions, deletions, and substitutions to these sequences may be made without significantly altering the activity of the molecules and are hence within the scope of the invention; and C, G, A, and U represent cytidine, guanosine, adenosine and uridine nucleotides,

respectively. The nucleotides in each of the formulae I and II are unmodified or modified at the sugar, base, and/or phosphate as known in the art.

The paragraph on page 11, lines 26-28:

Figure 7 is a representative diagram of structural similarities between Class I (SEQ ID NO [146]**117**), (substrate SEQ ID NO [147]**103**) and Class VIII (SEQ ID NO [148]**121**), (substrate SEQ ID NO [149]**103**) enzymatic nucleic acid molecules of the invention.

The paragraph on page 39, lines 2-15:

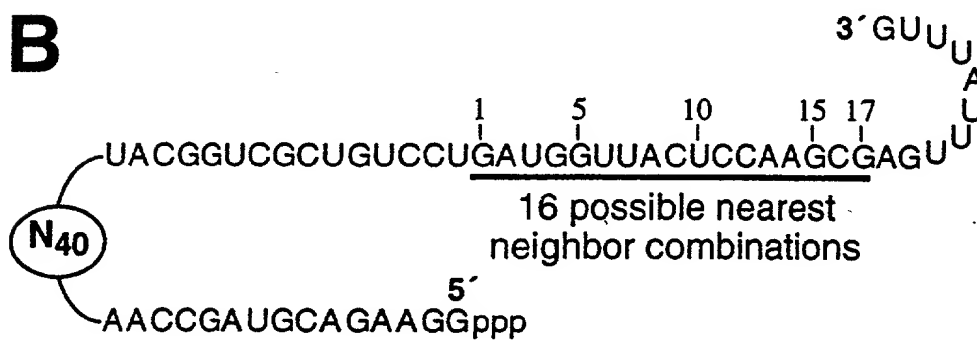
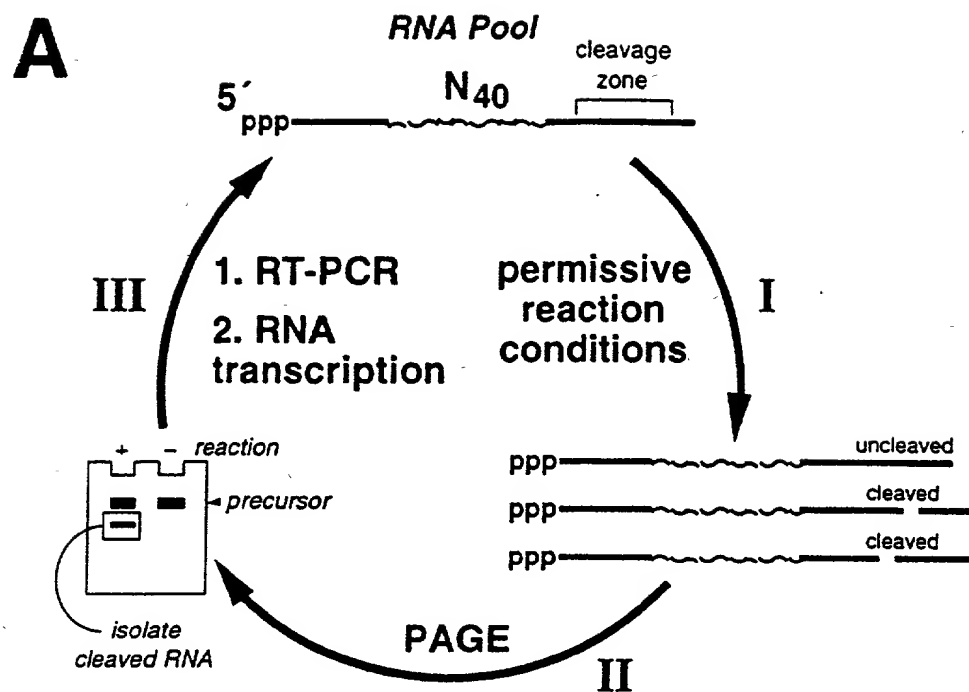
The initial population of RNA for *in vitro* selection was created by first generating a double-stranded DNA template for *in vitro* transcription. SuperScript® II reverse transcriptase (RT, GibcoBRL) was used to extend 280 pmoles of the DNA oligonucleotide "primer 1" (5'-GAAATAAACTCGCTTGGAGTAACCATCAGG-ACAGCGACCGTA-3') (SEQ ID NO [55]**99**); region representing 16 possible nearest neighbor combinations is underlined) using 270 pmoles of the template DNA (5'-TCTAATACGACTCACTATAGGAAGACGTAGCCAA-N₄₀TACGGTCGCTGTCCTG-3') (SEQ ID NO [56]**100**); T7 promoter is underlined and N represents an equal mixture of the four standard nucleotides). The extension reaction was conducted in a total of 50 µl containing 50 mM Tris-HCl (pH 8.3 at 23°C), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol (DTT), 0.2 mM each of the four deoxyribonucleoside-5' triphosphates (dNTPs), and 10 U µl⁻¹ RT by incubation at 37°C for 1 hr. The resulting double-stranded DNA was precipitated by the addition of 5 µl 3 M sodium acetate (pH 5.5) and 140 µl 100% ethanol and pelleted by centrifugation. This extension reaction provides ~10¹⁴ different template sequences.

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visualized and quantified using a Molecular Dynamics PhosphorImager®, and the gel region corresponding to the location of the desired RNA cleavage products was excised. The RNA was recovered from the excised gel by crush-soak elution followed by precipitation with ethanol. The selected RNAs were amplified by RT-PCR as described previously (10) using primers 1 and 2 (5'-GAATTCTAATACGACTCACTATAGGAA-GACGTAGCCAA-3') (SEQ ID NO [57]101); T7 promoter is underlined). The resulting double-stranded DNA from each round of *in vitro* selection was used to transcribe the RNA population for the subsequent round, in which all steps were conducted at ~1/10th the scale of G0. All other parameters of the selection process were maintained as in G0. Representative ribozymes from the populations derived from 6, 9, 12 and 15 rounds of selection were examined by cloning (TOPO®-TA cloning kit; Invitrogen) and sequencing (ThermoSequenase® kit; Amersham Pharmacia).

Figure 1



(SEQ ID NO 58)
(SEQ ID NO 102)

Figure 2

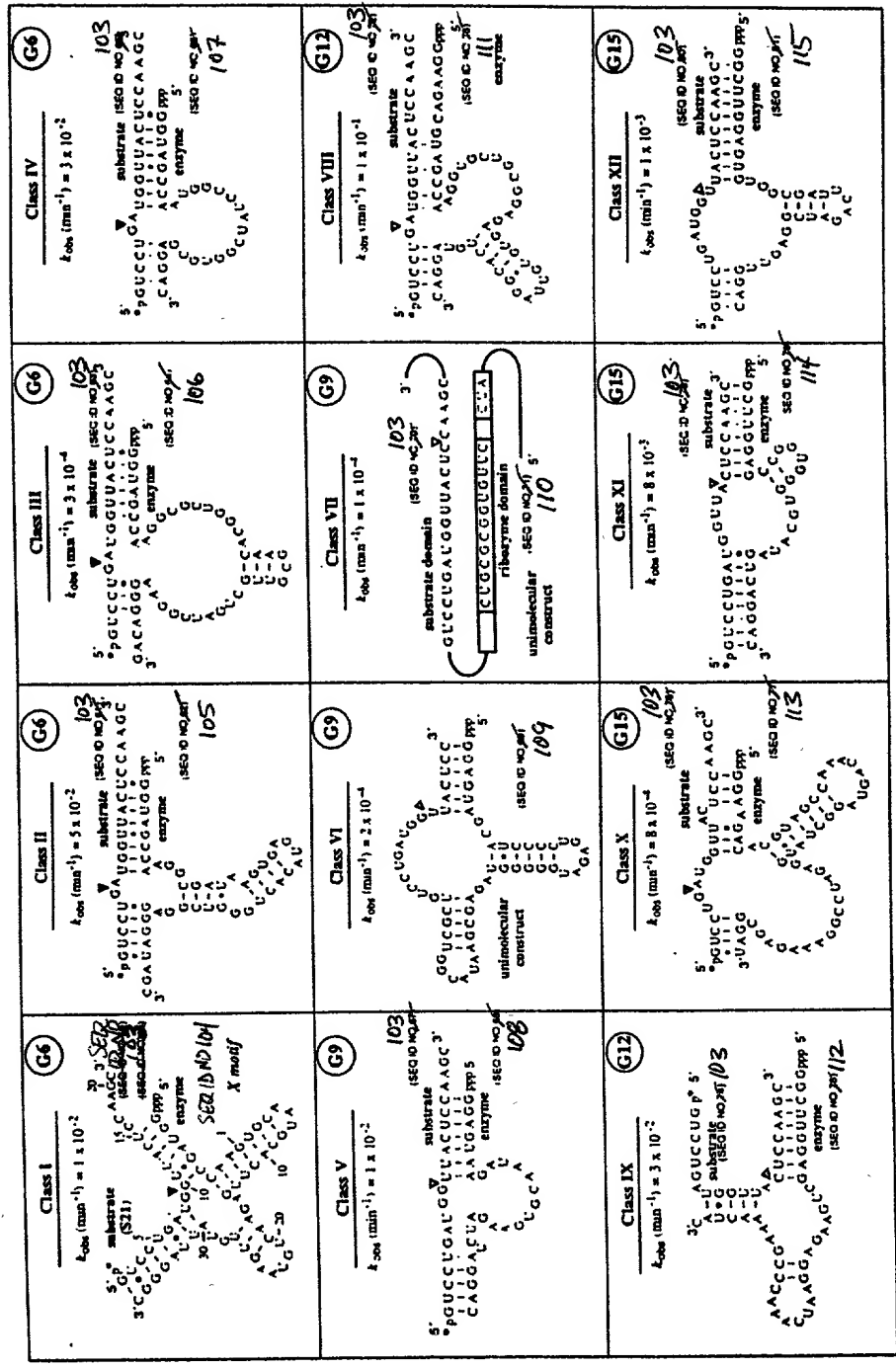
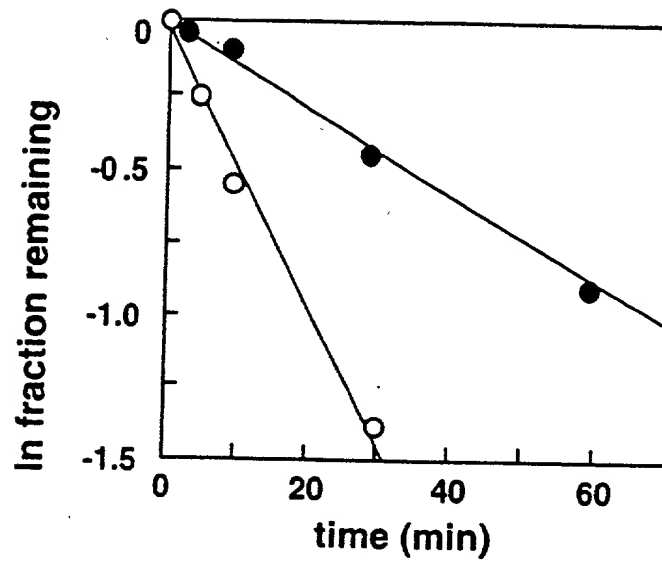
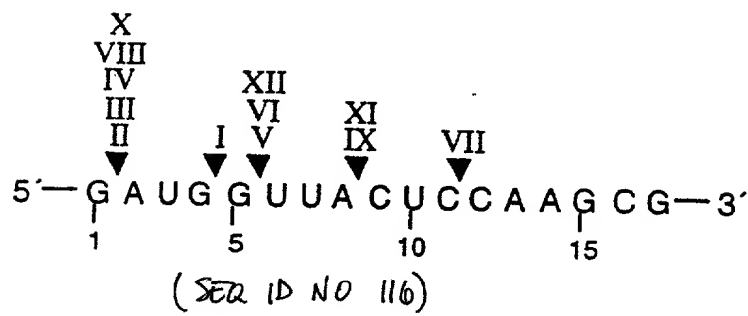


Figure 3

A



B



(SEQ ID NO 82)

Figure 4

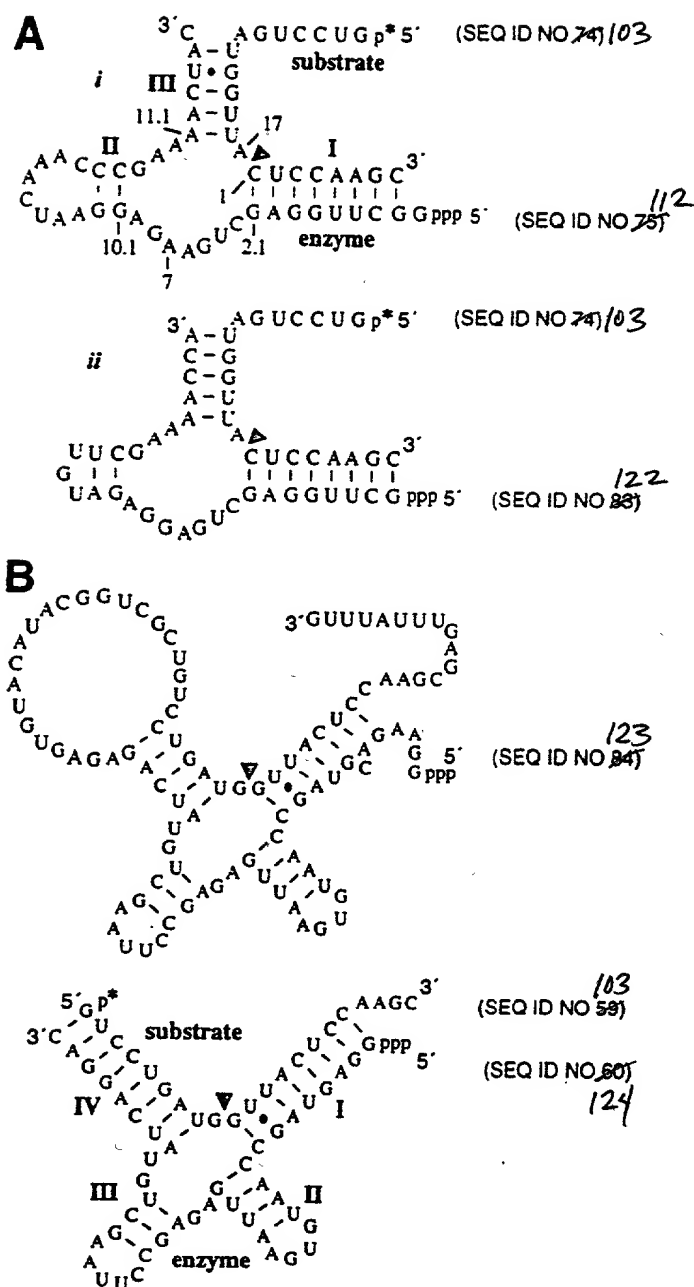
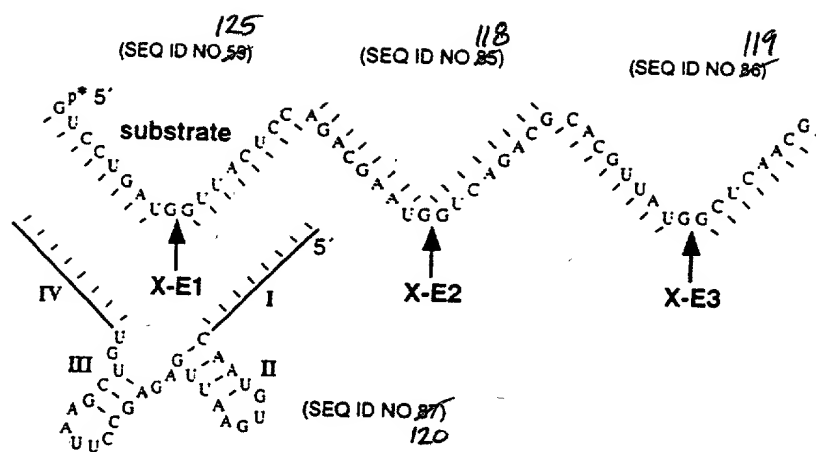


Figure 5



Rate for all 2'-O-Me enzyme with A14.1 = $\text{ribo } K_{\text{obs}} = 0.00008 \text{ min}^{-1}$

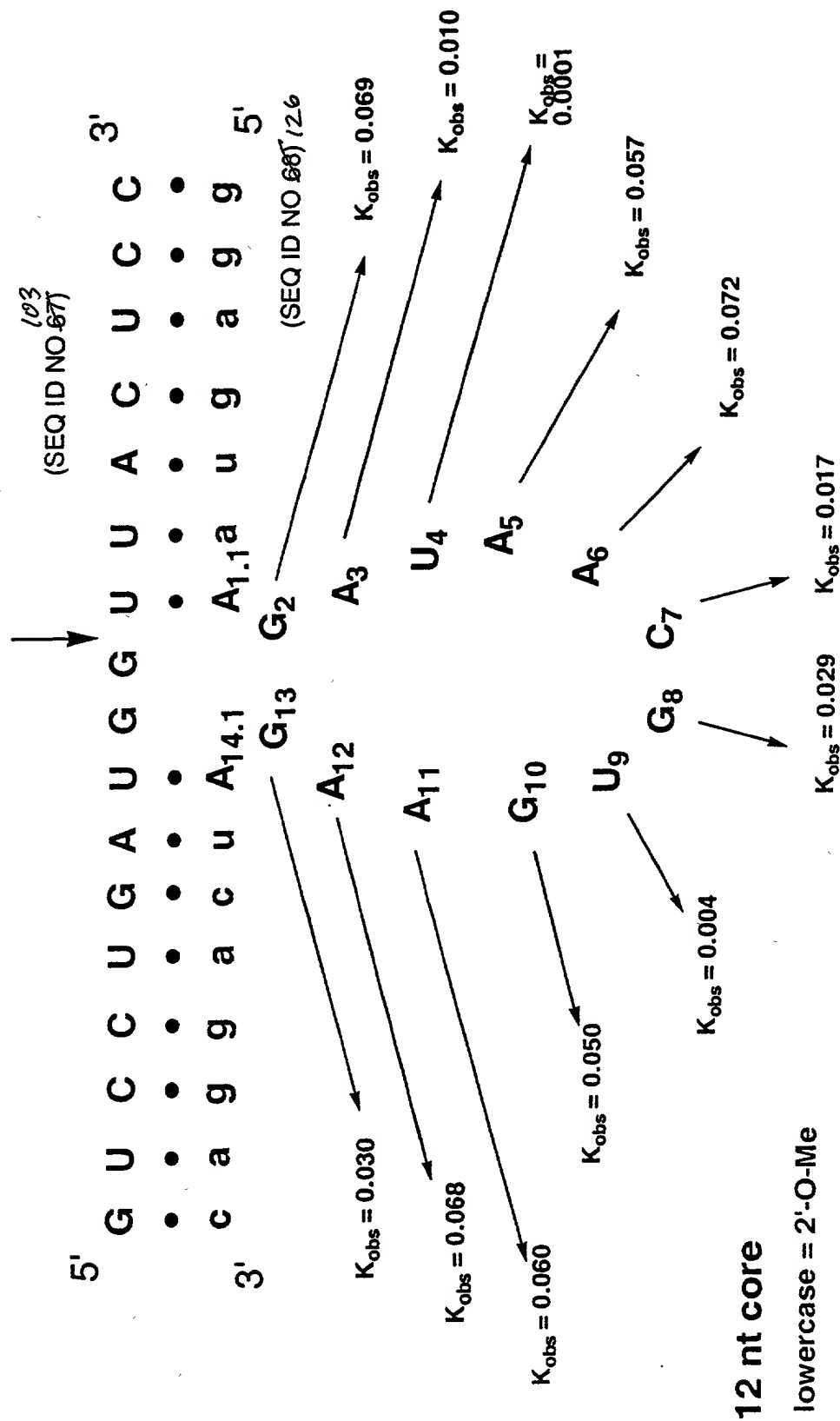
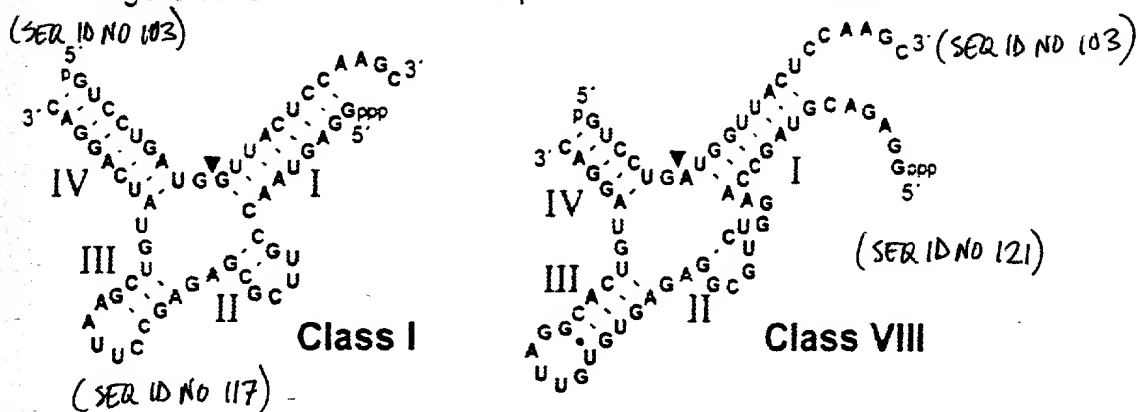
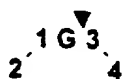


Figure 7: Class I and VIII Sequence and Structural Similarities



Class I motif cleavage site



1 = A or U
 2 = complementary to 1
 3 = C, A or U
 4 = complementary to 3